

Human T-cell responses to ragweed allergens: *Amb* V homologuesS.-K. HUANG & D. G. MARSH *Johns Hopkins Asthma and Allergy Center,
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SUMMARY

Specific IgE and IgG responses to highly purified *Ambrosia* (ragweed) allergens, *Amb a* V, *Amb t* V and *Amb p* V from the *artemisiifolia* (short), *trifida* (giant) and *psilostachya* (western) species are strongly associated with HLA-DR2 and Dw2 (DR2.2) in allergic Caucasoid individuals. To investigate the molecular basis of these HLA associations, we examined the human T-cell responses to these *Amb* V homologues using three *Amb a* V-specific, DR $\alpha\beta$ 1.2.2-restricted T-cell clones from an atopic patient. We first examined the cross-reactivity of *Amb a* V-specific T-cell clones upon challenge with the *Amb a* V homologues, *Amb t* V and *Amb p* V, in the presence of autologous antigen-presenting cells (APC). Neither *Amb t* V nor *Amb p* V was able to stimulate the T-cell clones directly. However, both *Amb t* V and *Amb p* V specifically blocked, in a dose-dependent fashion, the ability of APC to present *Amb a* V to all three T-cell clones. Taken together, these results suggest that *Amb t* V and *Amb p* V possess distinct T-cell epitopes, but that all *Amb* V homologues share similar or identical regions (agretope) interacting with the DR $\alpha\beta$ 1.2.2 (DR $\alpha\beta$ 1501) heterodimer. The agretope was potentially localized to a 14-residue C-terminal *Amb a* V peptide (with Ala–Cys substitutions), which was able to block presentation of native *Amb a* V by the APC to the T-cell clones.

The activation of helper/inducer T lymphocytes requires the co-recognition of antigen in the context of a class II major histocompatibility MHC (Ia) molecule, mediated through the trimolecular interaction between a clonotypic T-cell receptor (TcR), an antigen (Ag) peptide, and an Ia molecule.^{1–3} Immunogenic peptides thus possess two functional sites—a region that interacts with the TcR (epitope) and a region that binds to Ia (agretope). This functional diversity has been defined, primarily, in the murine model from studies of competition between antigenic peptides and their synthetic analogues in direct Ia binding or Ag presentation to cloned T cells. Analyses of the respective peptide sequences suggested that amphipathicity may be one of the important structural properties of Ia/T-cell recognition sites.^{3–5} Very little is known, however, regarding the structural features of allergen-derived peptides bridging the TcR and Ia molecules, despite the fact that Ag-specific T-cell activation is crucial in the regulation of Ab synthesis, including IgE,⁶ as well as chronic allergic inflammation.⁷

We have previously demonstrated that human immune responsiveness towards three cystine-rich ragweed allergens, *Amb a* V (MW = 5000), *Amb t* V (MW = 4400) and *Amb p* V (MW = ca. 5000), is associated with HLA-DR2.2,⁸ and our T-cell studies have shown that DR $\alpha\beta$ 1.2.2 (or the closely related DR $\alpha\beta$ 1.2.12) is functional in controlling T-cell responses to *Amb a* V (S. K. Huang *et al.*, *Eur. J. Immunol.*, in press). Although *Amb a* V and *Amb t* V show 45% amino acid sequence identity, they are virtually non-cross-reactive antigenically.⁹ *Amb a* V and

Amb p V show marked cross-reactivity in certain atopic patients.¹⁰ We hypothesized that the concordant responsiveness to *Amb a* V/*Amb p* V on the one hand and *Amb t* V on the other hand may result not simply from antigenic cross-reactivity, but rather may reflect concordance of immune recognition of similar or identical binding sites to the same Ia molecule. We have utilized three *Amb a* V-specific, DR $\alpha\beta$ 1.2.2-restricted T-cell clones to test this hypothesis and to identify the potential MHC binding site of the *Amb a* V.

Amb a V-specific T-cell clones were derived from peripheral blood mononuclear cells (PBMC) from a ragweed-allergic individual following periodic *Amb a* V plus recombinant interleukin-2 (rIL-2) stimulation, as described elsewhere (S. K. Huang *et al.*, *Eur. J. Immunol.*, in press). The three CD4⁺ *Amb a* V-specific T-cell clones (AP1.2, AP6, and AP9) thus generated were found to be restricted by the DR $\alpha\beta$ 1.2.2 (or the closely related DR $\alpha\beta$ 1.2.12) heterodimer. *Ambrosia* (ragweed) allergens *Amb a* V, *Amb t* V and *Amb p* V were isolated and purified from short, giant and western ragweed pollens, respectively.^{11–13} The protein sequences of *Amb a* V and *Amb t* V are known and show 45% homology (Fig. 1). *Lol p* III was isolated and purified from *Lolium perenne* (rye grass) pollen.¹⁴

We first examined whether *Amb t* V and *Amb p* V could directly stimulate *Amb a* V-specific T-cell clones. As shown in Fig. 2, neither *Amb a* V homologue in the presence of APC was able to stimulate the T-cell clones directly at Ag concentrations ranging between 0.1 μ g/ml and 10 μ g/ml. We then analysed whether *Amb t* V or *Amb p* V was able to inhibit *Amb a* V presentation, presumably by providing class II MHC binding sites which compete with the respective *Amb a* V MHC binding



Figure 1. Comparison of the amino acid sequences of the homologues, *Amb a V* and *Amb t V*. The sequences have been aligned to optimize homology, particularly with respect to the alignment of the cystine (C) residues. Homologous residues are boxed. The region of 10 amino acids, residue numbers 17–26, which shows homology in the two *Amb V* molecules is 'amphipathic'.¹⁵

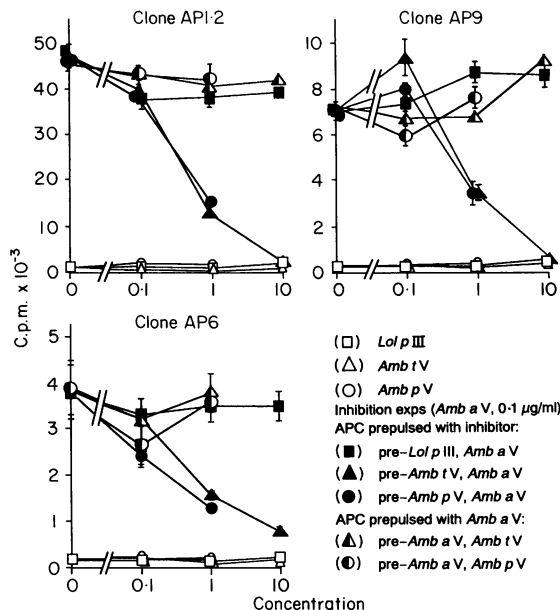


Figure 2. Inhibition of *Amb a V* presentation by *Amb t V* and *Amb p V*. Direct T-cell stimulation with *Amb t V* or *Amb p V* (open symbols), inhibition of *Amb a V* presentation by prepulsing APC with *Amb t V* or *Amb p V* (closed symbols), and APC prepulsed with *Amb a V* (half-closed symbols). *Lol p III* was used as the control Ag. For direct stimulation with Ag, T cells (5×10^4 cells/ml) were stimulated in triplicate with or without Ag in the presence of irradiated (5000 rads) autologous PBMC (5×10^5 cells/ml) for 3 days. Varying concentrations of highly purified *Amb V* allergens (*Amb a V* or *Amb t V* or *Amb p V*; or a control Ag, *Lol p III*) were added at the initiation of the culture. For blocking experiments, autologous APC (5×10^5 cells/ml) were preincubated with or without each of the *Amb a V* homologues (or *Lol p III* as control Ag) at 0.1–10 μg/ml in culture medium for 10 hr at 37° and washed three times. The prepulsed APC (1×10^5 cells/well) were then cultured with cloned T cells (1×10^4 cells/well) in the presence or absence of *Amb a V* (0.1 μg/ml) for 60 hr. Reciprocal experiments were run in parallel wherein autologous APC were prepulsed with 0.1 μg/ml of *Amb a V* followed by culturing with T cells and each of the homologues. The cell cultures were then pulsed with 1 μCi of [^{3} H]thymidine (Du Pont, Wilmington, DE), harvested 18 hr later, and counted. Results are expressed as [^{3} H]thymidine incorporation (mean c.p.m. $\pm$ SE) from triplicate cultures.

region. When preincubated with autologous APC (for 10 hr followed by extensive washing), both *Amb t V* and *Amb p V* could inhibit, in a dose-dependent manner, the ability of APC to present *Amb a V* to the three T-cell clones (Fig. 2; for methods, see Fig. 2 legend). The inhibitory functions of both *Amb t V* and *Amb p V* molecules were specific, since an unrelated control Ag, *Lol p III*, had no effect on cloned T-cell responses to *Amb a V*, even at a 100-fold higher concentration. Furthermore, neither *Amb t V* nor *Amb p V* was cytotoxic; each Ag lost its inhibitory

function when autologous APC were prepulsed with a suboptimal dose (0.1 μg/ml) of *Amb a V* (Fig. 2), and neither Ag was able to suppress the PHA-stimulated responses of the cloned T cells (data not shown). Either inhibitor, when added at the initiation of the culture, also blocked *Amb a V* presentation, although at a one-log higher concentration (data not shown).

Two possible explanations for this inhibition are that there is competition either at the level of antigen processing for the available pool of intracellular proteolytic enzymes, or at the level of antigen presentation by Ia molecules. We favour the latter explanation, since an unrelated antigen, *Lol p III*, showed no inhibition even at 100 times higher concentration. We are aware of the possibility that both *Amb a V* homologues, following preincubation with APC, may induce a state of T-cell 'anergy' to subsequent challenge with Ag, because of the lack of a 'secondary signal' from pretreated APC. However, our data show that T cells remained responsive to PHA in the presence of either *Amb t V*- or *Amb p V*-treated APC; and the fact is that both *Amb t V* and *Amb p V* are, themselves, active in stimulating immune responses (*in vivo* and presumably *in vitro*). Our data would, therefore, suggest a concordant recognition of similar or identical MHC binding sites on *Amb V* homologues by the DR $\alpha\beta$ 2.2 heterodimer. A definitive answer to this proposal awaits comparative studies using *Amb t V*- and *Amb p V*-specific T-cell clones.

To investigate the nature of the *Amb a V* MHC binding site, we analysed the ability of synthetic peptides either to stimulate cloned T cells or compete with *Amb a V* presentation. Except for the Ala–Cys substitutions, the two peptides, 12–26 and 31–44, correspond to portions of the *Amb a V* molecule that show strong homology with *Amb t V*; furthermore, there is evidence for amphipathicity within the regions 12–26 for both *Amb a V* and *Amb t V*.¹⁵ Our results show that both peptides, 12–26 and 31–44, were not stimulatory for all three T-cell clones tested at concentrations ranging from 0.1 to 10 μM (data not shown). Only the C-terminal peptide 31–44 was able to compete, in a dose-dependent manner (0.5 and 5 μM; or 0.825 μg/ml and 8.25 μg/ml), with *Amb a V* presentation (Fig. 3), localizing the MHC binding site of *Amb a V* to this region.

It is likely that the Ala–Cys substitutions in synthetic peptide 31–44 would alter the local conformation of native peptide sufficiently to result in the loss of the T-cell epitope. In fact, our preliminary experimental data showed that the reduced and alkylated *Amb a V* (followed by HPLC purification) was no longer stimulatory, suggesting the importance of cystine residues in the class II-restricted Ag recognition by the TcR. As a corollary, the importance of cystine residues in T-cell recognition in the mouse has been documented in the case of insulin¹⁶ and apamine.¹⁷

Further experiments are in progress to identify more definitely the sites interacting with Ia and TcR using proteolytic *Amb V* fragments. Alternatively, we will use the peptides derived from *Amb V* cDNA clones, in conjunction with site-directed

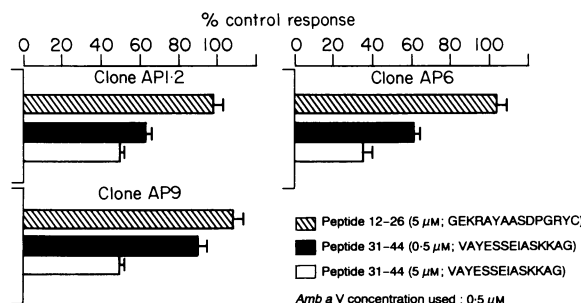


Figure 3. Inhibition of *Amb a V* presentation by *Amb a V* peptide analogues with alanine substitutions for cysteine. For peptide competition assays, varying concentrations of each peptide analogue were added at the initiation of the culture in the presence of *Amb a V*. The culture time period and conditions were the same as described in the legend to Fig. 1. Two peptides representing the *Amb a V* sequences, 12-26 and 31-44, were synthesized.¹⁸ The first peptide, 12-26, was synthesized with two Ala-Cys substitutions at positions 18 and 19; the second, 31-44, with three Ala-Cys substitutions at positions 32, 39 and 43. These data were expressed as follows: percentage of control response = $100 \times [(\text{c.p.m. of culture with } Amb a V \text{ and peptide} - \text{c.p.m. of culture without Ag}) / (\text{c.p.m. of culture with } Amb a V - \text{c.p.m. of culture without Ag})]$. The mean c.p.m. of culture with *Amb a V*: 9181 ± 201 ; and without Ag: 180 ± 45 . The mean c.p.m. of cultures with either peptide 12-26 or peptide 31-44 alone were not significantly different from background c.p.m. in all three T-cell clones tested.

mutagenesis experiments, to circumvent the problem of the high proportions of S-S bonds in these molecules.

The finding that a peptide analogue (peptide 31-44) of *Amb a V* is able to inhibit the T-cell responses to the native protein is of particular interest concerning its potential clinical implications. By employing a similar approach, it may be possible to construct a cocktail of allergen fragments, such as those described for the *Amb a V*-peptide with alanine substitutions. The fragments thus generated are potentially capable of blocking the T-cell responses to the native allergens, thereby providing a new form of immunotherapy for allergic disease.

Taken together, these studies serve to validate the use of allergy as a model for exploring the molecular basis of human immune responsiveness and its causal relationship to an immunological disease. The highly purified, well-defined *Amb V* homologues provide an optimal system for such studies.

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